EXTENDED EXPERIMENTAL PROCEDURES

HIV-1 gp140 protein production

The production of pre-fusion closed trimers from diverse HIV-1 strains has been an obstacle to structural and immunogenicity studies. One clade A strain, BG505 (Wu et al., 2006), stabilized by a disulfide (SOS) (Binley et al., 2000), isoleucine to proline mutation (IP) (Sanders et al., 2002), and truncation at residue 664 (Julien et al., 2013), has been shown to form stable soluble trimer (Sanders et al., 2013).

Plasmids encoding HIV-1 gp140 Env designs were screened by micro-transfection in 96-well format for expression as described in (McLellan et al., 2013a). By combining plasmids for scFv VRC01 or Fab heavy and light chains from VRC01 with JR-FL or BG505 gp140 constructs, production of stable pre-fusion HIV-1 trimers was confirmed by ELISA binding with quaternary antibodies PGT145 and CAP256-VRC26.09. Mutations that allow for efficient formation of a disulfide bond at the interface of VRC01 antibody (mutations $A60C_{HC}$ or $R61C_{HC}$) and gp140 ($G459C_{gp120}$) were identified from analyzing the gp120-VRC01 complex (PDB IDs 4LSS, 4NCO, more) with the program Disulfide by Design 2.0 (Craig and Dombkowski, 2013) (**Figure S1A, B**). Co-expression of $A60C_{HC}$ in the antigen-binding fragment (Fab) of antibody VRC01 along with the $G459C_{gp120}$ mutant BG505 IP.664.T332N resulted in homogenous disulfide bond formation between antibody and trimeric Env. SDS-PAGE analysis revealed the gp120-gp41 protease site to be fully cleaved (**Figure S1C, D**). A variety of Envs from diverse HIV-1 strains were then chosen based on neutralization sensitivity to both VRC01 and PGT145, SOSIP and $G459C_{gp120}$ mutations were introduced, terminating at either residue 664_{gp41} or 665_{gp41}, and co-expressed in HEK 293 GnTI⁻ cells with the single chain

variable domains (scFv) or Fab of VRC01 with $A60C_{HC}$ mutation. The expression in GnTI⁻ cells resulted in N-linked glycans being solely of the high mannose character, as opposed to the more diverse glycan reported for BG505 SOSIP (Behrens et al., 2016). Thirteen purified complexes of scFv VRC01 with HIV-1 trimers from clades A, B, C, D, G, CRF_AE and A/G/I yielded fully cleaved molecules (**Figure S1E-H**), which eluted on size-exclusion chromatography at an elution volume corresponding to ~500 kDa, and eleven of these appeared homogenous by negative-stain electron microscopy (**Figure S1I**). Antigenically, these VRC01 trimer complexes bound trimer apex quaternary antibodies PG9, PGT145, and CAP256-VRC26.09, in a manner consistent the neutralization sensitivity of the strains to these antibodies (**Figure S1J**). DNA plasmids used for co-transfection of a 1 liter cell culture (HEK 293T GnTI⁻ or EXPI cells) were mixtures typically of 333 µg gp140, 333 µg furin, 167 µg VRC01 light chain and 167 µg VRC01 heavy chain Fab. For $A60C_{HC}$ scFv VRC01 co-expression, 333 µg of the plasmid was used in place of the heavy and light VRC01 antibody Fab plasmids as above. In the scFv construct the light chain was linked to the heavy chain with a $(G_4S)_3$ linker between VRC01 residues 106_{LC} and 1_{HC}. A C-terminal, thrombin-cleavable, Ni^{2+} resin capture purification tag (GGLVPRGSHHHHHHHH) was added after $A60C_{HC}$ scFv VRC01 residue 111_{HC}. The VRC01 Fab co-expression constructs had the same tag on the VRC01 heavy chain following position 218_{HC}, however for all structures reported here, the $A60C_{HC}$ scFv VRC01 construct was used.

For gp140 constructs, the protease cleavage site between gp120 and gp41 was mutated (HXB2 sequence REKR) to RRRRRR and either the IP mutation alone or the SOSIP mutations (Binley et al., 2000; Sanders et al., 2002) were introduced in addition to a thrombin cleavable Strep-tag II tag (GGGLVPRGGGGSAWSHPQFEK) following either position 664 (including JR-FL) or 665 (including X1193.c1) of gp140.

HEK 293T GnTI$^{-/-}$ cultures were co-transfected using TrueFect-Max transfection reagent and fed with fresh 293FreeStyle media (Life Technologies) 4 h post-transfection and with HyClone SFM4HEK293 enriched medium (HyClone) containing valproic acid (4 mM final concentration) 24 h after transfection. Cultures were then incubated at 33º C for 6 days, supernatants clarified by centrifugation and 0.22μm filtration and HIV-1 Env trimer-VRC01 complexes purified by sequential Ni^{2+} (cOmplete His-Tag Purification Resin, Roche) and Strep-Tactin Superflow purification steps. Purification tags were removed by an overnight digestion with thrombin (Novagen) at 1.32 U/ml and Env trimer-VRC01 complexes were purified further by application and elution from a Superdex 200 column equilibrated with PBS.

For BG505 SOSIP.664 production, the protein was produced as described previously (Pancera et al., 2014). Briefly, BG505 SOSIP.664 DNA plasmid was co-transfected with furin plasmid DNA at a ratio of 3:2 in HEK 293T GnTI$^-$ cells using TrueFect-Max transfection reagent (United Biosystems) according to manufacturer's protocol and the cells were cultured for 6 days at 33º C. Supernatants were harvested and clarified by centrifugation and filtration through a 0.22μm filter and passed over an immobilized 2G12 column. After washing with PBS, bound protein was eluted with 3M MgCl$_2$, 10 mM Tris pH 8.0. The eluate was concentrated to less than 2 ml with a Centricon-70 concentrator and applied to a Superdex 200 column equilibrated in 5 mM HEPES, pH 7.5, 150 mM NaCl and 0.02% azide. The peak corresponding to trimeric HIV-1 Env was pooled and kept on ice until required.

Fab expression and purification

PGT122 and 35O22 IgGs were expressed as previously described (Pancera et al., 2014). Heavy chain plasmids containing the HRV3C cleavage site after Lys 218 in the hinge region

were co-transfected with light chain plasmids in EXPI (35O22) or HEK 293S GnTI⁻ (PGT122) cells using TrueFect-Max transfection reagent (United Biosystems) according to manufacturer's protocol. Cultures were fed with fresh 293FreeStyle media (Life Technologies) 4 h post-transfection and then with HyClone SFM4HEK293 enriched medium (HyClone) containing valproic acid (4 mM final concentration) 24 h after transfection. Cultures were incubated at 33º C for 6 days, and supernatants harvested, clarified by centrifugation and filtration and passed over a protein A affinity column. After a PBS wash and low pH elution, the eluate was pH neutralized with 1M Tris pH 8.0. Fabs were obtained using HRV3C digestion overnight at room temperature and collected from flow-through from protein A column to remove Fc fragments. Fabs were then further purified through a Superdex 200 column in PBS.

Fab complex preparation

PGT122 and 35O22 Fabs were each added to a solution of purified gp140 trimers in >2-fold molar excess overnight at room temperature. The complexes was then concentrated and purified over gel filtration equilibrated in 5 mM HEPES, pH 7.5, 150 mM NaCl, 0.02% azide (**Figure S1K**). Fractions were pooled, concentrated to 6-10 mg/mL and used for crystallization screening or kept on ice until further use.

Crystallization screening

The HIV-1 trimer complexes were screened for crystallization using 572 conditions from Hampton, Wizard and Precipitant Synergy (Majeed et al., 2003) screens using a Cartesian Honeybee crystallization robot as described previously (McLellan et al., 2011) and a mosquito robot using 100 nl of reservoir solution and 100 nl of protein solution. For the BG505

SOSIP.664-PGT122-35O22 complex, a 10% v/v additive screen (Hampton Research) was performed based on the previously reported condition for crystallizing the partially deglycosylated ternary complex: 16% isopropanol, 5.32% PEG 1500, 0.2 M Li_2SO_4, 0.1M Na acetate pH 5.5. Crystals grew optimally as rods in 0.18 M Li_2SO_4, 4.79% PEG 1500, 14.4% isopropanol, 10 mM yttrium chloride and 0.09 M sodium acetate pH 5.5. Crystals of fully glycosylated 293T-expressed BG505 SOSIP bound to PGT122 and 35O22 Fabs were also obtained; however the quality of these crystals needed further optimization to be sufficient for atomic-level analysis. For both the JR-FL-scFv VRC01-PGT122-35O22 and X1193.c1-scFv VRC01-PGT122-35O22, crystals were identified in the 100 nl screen with 4.95% isopropanol, 8.25% PEG 3350 and 0.2M ammonium citrate pH 4.5 and hexagonal rods grew to maximum dimensions of 150 μm × 150 μm × 300 μm. We were also able to obtain crystals containing clade C trimers using the PGT122 and 35O22 lattice, but the crystal size and resolution obtained were of lower quality than with the BG505, JR-FL and X1193.c1 complexes. Crystals were cryoprotected in solutions of 20% ethylene glycol, maintaining the mother liquor components at the original concentrations, and flash-frozen in liquid nitrogen. Data were collected at a wavelength of 1.00 Å at the SER-CAT beamline ID-22 (Advanced Photon Source, Argonne National Laboratory).

X-ray data collection, structure solution and model building

Crystals for X1193.c1-scFv VRC01-PGT122-35O22 were spacegroup $P6_3$, and diffraction extended to 3.1 Å resolution along the *c* axis (c-axis $I/\sigma I > 3.0$) and to lower resolutions along *a* and *b* axes, resulting in an overall dataset resolution with $I/\sigma I$ of ≥ 2.0 of 3.4 Å (**Table S1**). Structure solution by molecular replacement followed by rigid body fitting

revealed extensive glycosylation; rebuilding to optimize geometry and glycan stereochemistry yielded R_{work}/R_{free} values of 21.4%/27.2% (**Table S1**, **Figure S1L-O**). Diffraction from JR-FL-scFv VRC01-PGT122-35O22 and BG505-PGT122-35O22 crystals were similarly anisotropic to the X1193.c1 data and extended to nominal resolutions of 3.7 Å. Molecular replacement and refinement led to R_{work}/R_{free} of 25.3%/30.9% and 26.0%/30.7% for JR-FL and BG505, respectively (**Tables S1 and S3**). Interestingly, despite general similarity of the lattice, the crystallizing antibodies did show strain-dependent variation, especialy with N137, a glycan known to be important in PGT122 recognition (Garces et al., 2015; Garces et al., 2014). N137 was disordered in JR-FL, ordered in BG505 and displaced by a V1 extension in X1191.c1 (**Figure S2E**).

Diffraction data were processed with the HKL2000 suite (Otwinowski and Minor, 1997). The data were corrected for anisotropy by http://services.mbi.ucla.edu/anisoscale/ with truncations to 3.4 Å, 3.4 Å, 3.1 Å (X1193.c1) and 3.7 Å, 3.7 Å, 3.1 Å (JR-FL and BG505) along a, b, and c axes, respectively. Structure solution was obtained with Phaser (McCoy et al., 2007) using BG505 SOSIP.664-PGT122-35O22 (PDB ID: 4TVP) (Pancera et al., 2014), as search model. Protein polypeptide chains for gp120 and gp41 were re-built using Autobuilder (Terwilliger et al., 2008) to eliminate any glycan model bias and in the absence of glycan coordinates revealed extensive and unambiguous carbohydrate electron density near the protein surface from Fo-Fc difference maps. Refinement was carried out with Phenix (Adams et al., 2004) and Buster (Bricogne, 2011). Model building was carried out with Coot (Emsley and Cowtan, 2004). Carbohydrates were modelled into Fo-Fc maps and further refined using Fo-Fc, $2F$o-Fc and feature-enhanced maps (Afonine et al., 2015). Glycan geometries and real-space correlation coefficients were determined using the program Privateer, confirming positive real-

space correlation coefficients for all modelled glycans and 100% $^4C_1$ carbohydrate geometry for all sugar residues in the three structures (Agirre et al., 2015). The crystallographic refinement of glycans enabled dominant glycan conformations and estimations of glycan type based on density-fitting; however, the B-factors indicate that flexibility inherent to HIV-1 Env glycans might result in modelled structures that could vary from those present here. The Ramachandran plot as determined by MOLPROBITY (Davis et al., 2004) showed >89.8% of all residues in favored regions and >99.6% of all residues in allowed regions. Data collection and refinement statistics are shown in Table S1. Surface area calculations were performed using the program ASC (Eisenhaber, 1993).

Glycan analysis

Glycosylation profiles of N-linked glycans of purified Env proteins and antibodies were determined by hydrophilic interaction liquid chromatography-ultra performance liquid chromatography (HILIC-UPLC) as previously described (Pritchard et al., 2015). Briefly, glycoprotein bands were excised from Coomassie blue stained SDS-gels and released by addition of protein N-glycosidase F (PNGase F) at 5000 U/ml and incubation at 37 ˚C for 16 h, according to the manufacturer's instructions (New England Biolabs). Released glycans were eluted out of the gel bands by washing with water, dried down using a SpeedVac concentrator, labelled with 2-aminobenzoic acid (2-AA) and purified. Fluorescently labelled glycans were resolved by HILIC-UPLC using a 2.1 mm × 10 mm Acquity BEH Amide column as previously described. Empower 3 software was used for peak integration.

Saturation-Transfer Difference Nuclear Magnetic Resonance

Binding of VRC01 to $Man_9GlcNAc_2Asn$ was detected by NMR using saturation transfer methods as described (McLellan et al., 2011; Meyer and Peters, 2003). In this method, ligand protons that were within 5 Å of the protein or receptor in the bound state showed STD enhancement. Data were acquired on a Bruker Avance 600 MHZ instrument equipped with a triple resonance cryo-probe with *z*-axis gradients. Topspin 2.1 software was used to process and analyze the NMR data. The saturation pulse consisted of a train of 50 msec EBURB2 pulses (BW=100 Hz, B1=82 Hz) separated by 1 msec for a total saturation time of 2 sec with on and off resonance pulses applied at -1.5 and -20 ppm, respectively. A 10 KHz spin lock was applied for 10 msec before 3-9-19 water suppression and 1s of data acquisition (sw = 15ppm, 16k points, 1 and 2 msec post-acquisition purge pulses at 17 kHz, relaxation delay = 2.5s, 4 K scans). The saturation transfer difference spectrum was obtained by subtracting the on-resonance spectrum from the reference spectrum. Samples were prepared in 20 mM sodium phosphate buffer containing 50 mM sodium chloride at pH 6.8. All experiments were carried out at 298 K.

Nearest Inter-*N*-linked glycan sequon analysis

The distances for each *N*-linked glycan sequon and nearest *N*-linked glycan sequon were calculated for various viral glycoproteins. Asparagine Nδ2 atoms were used to calculate the distances. We analyzed respiratory syncytial virus (RSV) fusion glycoprotein (McLellan et al., 2013b), influenza virus hemagglutinin (HA) (Stevens et al., 2006) and the Ebola virus core envelope glycoprotein (GP) (Lee et al., 2008), where glycan sequons were considerably more sparse than with HIV-1 (**Figure 3B**). Higher sequon density was observed with SIV gp120 (Chen et al., 2005), HCV E2 core (Kong et al., 2015), and the Epstein-Barr virus gp350 (Szakonyi et al., 2006), though none of these displayed glycans with nearest neighbor distances

of less than 9 Å. The PDB IDs used for the non-HIV-1 Env glycoproteins were the following:

RSV fusion protein: 4JHW; influenza HA: 4M4Y; Ebola GP core: 3CSY; EBV-gp350: 2H6O;

HCV E2 core: 4MWF; SIV gp120: 2BF1.

Glycan modeling and molecular dynamics simulations

We constructed three all-atom fully glycosylated trimer models using the coordinates of

the BG505 x-ray structure (PDB ID: 5FYL) as a template. Any missing segments in the BG505

x-ray structure were modeled using LoopyMod (Soto et al., 2008) based on the native BG505

sequence. Missing side chains were modeled using the program SCAP (Xiang and Honig, 2001).

Using in-house software we modeled a mannose-5, mannose-7 and mannose-9 (Man-5, Man-7

and Man-9) sugar moiety at each N-linked glycosylation sequon along the BG505 protein

sequence. The resulting three models (Man-5, Man-7 and Man-9) were fully solvated in a water

box of dimensions $175 \times 175 \times 175$ Å^3 with a 17 Å padding. The system was neutralized by the

addition of NaCl at a concentration of 150 mM. The CHARMM36 force field was used for the

parameterization of the protein (including CMAP corrections) (Best et al., 2012) and the

mannose-9 (Guvench et al., 2011). TIP3P parameterization (Jorgensen, 1983) was used to

describe the water molecules.

All molecular dynamics (MD) simulations involving the three fully glycosylated trimer

models (Man-5, Man-7 and Man-9) were performed with ACEMD software (Harvey et al., 2009)

in explicit solvent on a METROCUBO workstation (https://www.acellera.com/products/GPU-

hardware-molecular-dynamics-metrocubo/). The system was minimized for 2000 steps, followed

by equilibration in the NPT ensemble for 50 ns at 1 atm and 300 K using a time-step of 2 fs, rigid

bonds, cutoff of 9 Å and PME for long range electrostatics. During the equilibration, heavy

protein atoms were constrained by a 1 kcal/molÅ^2 spring constant and slowly relaxed over the first 5 ns, and the protein was allowed to move freely thereafter. The system was then simulated for 500 ns under the NVT ensemble using ACEMD in the NVT ensemble using a Langevin thermostat with damping of 0.1 ps^{-1} and hydrogen mass repartitioning scheme to achieve time-steps of 4 fs.

Snapshots from each of the three the MD simulation were taken at 0.1 ns time intervals for structural analysis. To assess the internal geometry of each glycan moiety, we analyzed the distribution of GlcNAc-GlcNAc-β-mannose angles (kink angle) for each simulation. The kink angle is defined as the angle formed between the geometric centers of the first three saccharide rings originating from the Nδ2 atom of a glycosylated asparagine residue. We also assessed the distribution of glycan-glycan contacts by a defining a contact as any glycan that has 10 or more atoms within a distance of 5.0 Å to another glycan.

Glycan-antibody overlap analysis

For the glycan-antibody overlap analysis we determined the number of atoms from each glycan that occupied the same volume that would be potentially occupied by an antibody. We used 8 structures that were co-crystallized with gp120 (PDB IDs: 4JAN, 4JM2, 4YDJ, 4YE4, 3U2S, 5FYJ, 4P9H and 2NY7), two antibody structures that were co-crystallized an HIV-1 prefusion trimer (PGT122 and 35O22; PDB ID: 4TVP), one antibody structure docked onto a HIV-1 prefusion trimer using a cryoEM density map (PGT151; PDB ID: 4NUG) and one antibody structure co-crystallized with CD4 (PDB ID: 1GC1). Each co-crystallized structure was aligned to each of the three MD trajectories at 0.1 ns time points using TM-align (Zhang and

Skolnick, 2005). After superimposition, we determined all glycan atoms from the MD trajectory within 3.0 Å of the antibody or CD4 structures.

Antibody epitope definition and serological prevalence

The antibody-epitope residues are antigen residues that have at least one non-hydrogen atom within 5.5 Å of any antibody non-hydrogen atom. The chronic serological prevalence of HIV-1 nAbs based on analysis of serum neutralization of a panel of diverse HIV-1 isolates were adapted from (Pancera et al., 2014), with detailed method described in (Georgiev et al., 2013).

Structural analysis and the definition of crowded and dispersed glycans

N-linked glycosylation sequons in each of the x-ray structures that contained an attached oligosaccharide unit were labeled as occupied and used to determine the number of nearby sequons within an R angstrom spherical shell (where R ranged in length from 10-60 Å spaced at 5 Å intervals). The distance between occupied *N*-linked glycosylation sequons was measured between Nδ2 atoms on the associated asparagine residues. The total number of occupied sequons at each position along the three protein sequences was correlated with the total number of resolved oligosaccharide units for each spherical shell using a Pearson correlation coefficient (with a two-tailed test to determine the statistical significance). All statistical calculations were carried out using R (Core, 2013).

The number of neighboring glycans from each of the three x-ray structures was also used to determine if a glycan was either crowded or dispersed. We used a 2×2 contingency table where we defined groups as either crowded or dispersed depending on the number of neighboring sequons within a spherical shell of 50 Å and the number of attached saccharide

units. Using Fisher's Exact test, we sampled all integral values between 5-30 Å representing the spherical shell containing the number of neighboring sequons and 3-11 for the number of saccharide units attached to the sequon. We achieved optimal partitioning at a value of 15 Å and 4 saccharide units (according the computed p-value). Thus, any glycan with 15 or more neighboring sequons within a 50 Å shell was labeled as being crowded and any glycan within this same shell with fewer than 15 neighboring glycans was labeled as dispersed. Fisher's Exact test was calculated using R.

To assess the variability of M-group sequences on each of the three x-ray structures we threaded 2994 M-group Env sequences from all major clades from the "filtered web alignment" (without recombinants) from the Los Alamos HIV database (www.hiv.lanl.gov) onto each structure using the homology modeling package Nest with default settings (Petrey et al., 2003). calculated the number of glycan sequons within a 50 Å radius of each glycan sequon to compare these values to those found for BG505, JR-FL and X1193.c1 structures (Figure 4E, Figure S4E). The M-group crowded glycan proximal, dispersed glycan proximal, and no glycan residues were defined as follows. Only those N-glycan sequence motifs for which Asn was at a surface-exposed position (SASA > 0.25) in the native crystal structure used for threading were considered as active sequons. The surfaces for each of the M-group sequences using the structures threaded to the X1193.c1 crystal structure, and then each residue was classified according to the most prevalent type observed over all M-group sequences. Significant correlation was observed between the number of glycan neighbors observed for each crystallized strain and the median number of glycan neighbors for the M-group models (Pearson correlation coefficient r = 0.96–0.97, p = $9.9 \times 10^{-15} - 4.7 \times 10^{-12}$). Pearson correlation coefficients and associated p-values were calculated using the package Scientific Python (SciPy).

The crowded-glycan-proximal surface were defined as surface residues (residues with at least 25% surface accessibility) which have at least one heavy atom within 10 angstoms of any crowded glycan asparagine Nδ2 atom. The dispersed-glycan-proximal surface were defined as surface residues which have at least one heavy atom within 10 Å of any dispersed glycan asparagine Nδ2 atom and were not crowded-glycan proximal surface residue.

Fabrication of NHS-Glycan Microarray

The 40 glycans used in the array analysis are prepared by the modular synthesis method developed recently (Shivatare et al., 2016; Shivatare et al., 2013). All monovalent glycans were prepared in 10 mM concentration individually and served as mother solutions, which were diluted with printing buffer to prepare a working solution. Microarrays were prepared by printing (BioDot Cartesion Technologies) (Blixt et al., 2004) with robotic pin (SMP3: TeleChem International) and printed onto NHS-coated glass slides (Nexterion H, SCHOTT). Printed slides were allowed to react in an atmosphere of 80% humidity for one hour followed by desiccation overnight. Individual glycans were spotted with five replicas. The prepared slides were stored in a humidity controlled dry box before the binding assay. The HIV-1 broadly neutralizing antibodies were complexed with Donkey Anti-Human IgG added to the glycan array and incubated at 4°C overnight. Slides were scanned with a GenePix 4300A reader (Molecular Devices, USA) and analyzed with GenePix Pro 7.0 software (Molecular Devices, USA).

Antibody-Glycan Binding Assay

Glycan arrays were blocked with blocking buffer (Superblock, Thermo Fisher Scientific, USA) for 1 hour and washed with PBST buffer (PBS buffer with 0.05% Tween 20). The HIV-1

broadly neutralizing antibodies (25 µg/mL in BSA contained PBST buffer, 3% w/v) were pre-complexed with Donkey Anti-Human IgG (Alexa Fluor647 conjugated, Jackson ImmunoResearch, USA) in 1:1 ratio and incubated at 4°C for 30 minutes. The pre-complexed mixture was added to the glycan array and incubated at 4°C overnight. The slides were washed sequentially in PBST buffer (PBS and 0.05% Tween-20), and de-ionized water. Slides were spin-dried prior to scanning with a GenePix 4300A reader (Molecular Devices, USA) and analyzed with GenePix Pro 7.0 software (Molecular Devices, USA). The image resolution was set to 5 µm per pixel. Spots were defined as circular features with maximum diameter of 100 µm. The total intensity of fluorescence was calculated and illustrated with Prism 6.0 (Graphpad, USA). Error bars represent the average percentage error for all data points reported (**Figure S6F, G**).

Neutralization assays

Monoclonal antibody (mAb) neutralization was assessed in TZM-bl cells as described previously (Montefiori, 2009; Wu et al., 2010). Briefly, 293T cells were cotransfected by a pSG3ΔEnv backbone and a wildtype BG505, JR-FL and X1193.c1 or T278A mutant HIV-1 Env expression plasmids to produce Env-pseudotyped virus stocks. Viruses were mixed with 5-fold serially diluted mAbs starting at 50 µg/ml, and incubated at 37 °C for 1 hour before adding to the cells. After incubation at 37 °C for 48 hours, the supernatants were removed and the cells were lysed. Luciferase activity was measured. 50% inhibitory concentrations (IC_{50}) were determined as described (Wu et al., 2010).

SUPPLEMENTAL TABLES

Table S2. HIV-1-Env glycoprotein PDB coordinate analysis, related to Figures 1 and 2.

Env	Env construct	PDB ID	Glycan mass (Da)	Protein mass (Da)	Ratio glycan/protein	Reference
Domain	pfam00516 eODmV3	3TYG	2737.2	16461.9	0.17	Pejchal 2011 *Science*
	ZM109 V1V2	3U2S	1330.6	4306.8	0.31	McLellan 2011 *Nature*
gp120	HXBc2 gp120	1GC1	2445.1	30763.2	0.08	Kwong 1998 *Nature*
	93TH057 gp120	4JKP	3117.4	35788.3	0.09	Diskin 2013 *J Exp Med*
	YU2 gp120	4RQS	4752.1	30455.1	0.16	Kong 2015 *Proteins*
Trimer	KNH1144 SOSIP	4CC8	0.0	112377.8	0.00	Bartesaghi 2013 *NSMB*
	BG505 SOSIP	4NCO	23722.3	138631.8	0.17	Julien 2013 *Science*
	BG505 SOSIP	3J5M	0.0	137683.6	0.00	Lyumkis 2013 *Science*
	BG505 SOSIP	4TVP	10150.5	60557.8	0.17	Pancera 2014 *Nature*
	BG505 SOSIP	4ZMJ	4600.1	59931.4	0.08	Kwon 2015 *NSMB*
	BG505 SOSIP	5A8H	7983.8	95069.4	0.08	Scharf 2015 *Cell*
	BG505 SOSIP	5ACO	33416.9	179152.0	0.19	Lee 2015 *Structure*
	BG505 SOSIP	5C7K	12013.2	60067.6	0.20	Kong 2015 *Acta Cryst. D*
	BG505 SOSIP	5CEZ	7071.2	62490.9	0.11	Garces 2015 *Immunity*
	BG505 SOSIP	5CJX	13343.8	154545.9	0.09	Scharf 2015 *Cell*
	JR-FL deltaCT	5FUU	35960.2	167932.9	0.21	Lee 2016 *Science*
	X1193.c1 SOSIP	5FYJ	24596.5	66102.9	0.37	This study
	JR-FL SOSIP	5FYK	20300.7	63095.4	0.32	This study
	BG505 SOSIP	5FYL	18171.8	60557.7	0.30	This study

Table S3. Crystallographic data and refinement statistics, related to Figures 1 and 2.

HIV-1 gp140 trimer	Glycosylated X1193.c1 SOSIP.665 scFv VRC01, Fabs PGT122 & 35O22	Glycosylated JR-FL SOSIP.664 scFv VRC01, Fabs PGT122 & 35O22	Glycosylated BG505 SOSIP.664 Fabs PGT122 & 35O22
PDB ID	5FYJ	5FYK	5FYL
Data collection			
Space group	$P6_3$	$P6_3$	$P6_3$
Cell dimensions			
a, b, c (Å)	127.16, 127.16, 313.65	130.78, 130.78, 314.62	129.78, 129.78, 313.06
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	50.00 – 3.10 (3.55-3.41) (3.41-3.29) (3.29-3.19) (3.19-3.10)	50.00 – 3.10 (3.91-3.68) (3.68-3.49) (3.49-3.34) (3.34-3.21) (3.21-3.10)	50.00 - 3.10 (3.91-3.68) (3.68-3.49) (3.49-3.34) (3.34-3.21) (3.21-3.10)
R_{sym} or R_{merge}	0.118 (0.579) (0.797) (0.969) (1.00)	0.109 (0.380) (0.403) (0.496) (0.558) (0.784)	0.099 (0.475) (0.490) (0.635) (0.698) (0.748)
R_{pim}	0.075 (0.376) (0.533) (0.699) (0.842)	0.066 (0.237) (0.267) (0.355) (0.436) (0.714)	0.060 (0.231) (0.246) (0.329) (0.369) (0.434)
$I/\sigma I$	9.05 (2.00) (1.44) (0.95) (0.64)	7.76 (2.19) (1.51) (1.09) (0.80) (0.55)	4.82 (2.12) (1.59) (1.16) (0.71) (0.67)
Completeness (%)	99.7 (100) (100) (99.7) (97.1)	86.2 (53.2) (42.1) (32.0) (22.8) (13.7)	85.1 (56.2) (46.3) (38.0) (31.1) (21.6)
Redundancy	6.0 (6.1) (5.9) (5.4) (4.2)	3.7 (2.9) (2.5) (2.1) (1.8) (1.3)	5.0 (4.1) (3.8) (3.4) (3.1) (2.9)
Refinement			
Resolution (Å)	45.0-3.10	45.0-3.10	45.0-3.10
Reflections	42,755	31,882	32,566
R_{work}/ R_{free}	21.4/27.2	25.3/30.9	26.0/30.7
No. atoms			
Protein	13481	13285	11345
Carbohydrate	1935	1565	1388
Ligand/ion	46	-	-
Water	12	-	-
B-factors			
Protein	120	168	122
Ligand/ion	123	-	-
Carbohydrate	159	195	161
Water	34	-	-
R.m.s deviations			
Bond lengths (Å)	0.005	0.006	0.005
Bond angles (º)	1.060	1.081	1.001
Ramachandran statistics			
Allowed (%)	99.5	99.6	99.7
Favored (%)	90.6	89.9	89.9
$^4C_1$ carbohydrate geometry (%)	100	100	100
MolProbity all-atoms clashscore	4.38	5.76	5.21
MolProbity score	2.48	2.65	2.60

Table S4. Modelled glycan size and real space correlation coefficients (RSCC) for glycans, related to Figures 1 and 2.

X1193.c1			JR-FL			BG505		
Sequon	Glycan residues	RSCC	Sequon	Glycan residues	RSCC	Sequon	Glycan residues	RSCC
N88	8	0.81	N88	7	0.81	N88	7	0.73
N133	1	0.39	N135	1	0.50	N133	2	0.61
N142	1	0.29	N156	9	0.65	N137	7	0.52
N156	11	0.44	N160	7	0.34	N156	9	0.70
N160	9	0.44	N187	7	0.52	N160	8	0.55
N188	10	0.36	N241	4	0.70	N197	6	0.47
N197	3	0.68	N262	9	0.77	N234	5	0.67
N234	6	0.63	N276	8	0.72	N262	8	0.76
N241	7	0.60	N295	5	0.61	N276	5	0.43
N262	11	0.81	N301	8	0.60	N295	7	0.62
N276	9	0.62	N332	10	0.78	N301	8	0.59
N293	9	0.59	N339	8	0.56	N332	10	0.82
N301	6	0.74	N355	7	0.49	N339	1	0.60
N332	10	0.76	N362	4	0.69	N355	1	0.64
N344	7	0.48	N386	8	0.51	N363	3	0.71
N355	3	0.36	N392	6	0.44	N386	6	0.62
N386	8	0.51	N397	2	0.71	N392	6	0.34
N392	1	0.30	N448	6	0.71	N448	6	0.58
N413	10	0.53	N463	2	0.37	N611	1	0.30
N442	6	0.56	N611	3	0.45	N618	1	0.43
N464A	2	0.04	N616	1	0.54	N637	3	0.29
N611	2	0.71	N625	1	0.59			
N616	2	0.59	N637	1	0.08			
N625	3	0.59						
N637	2	0.71						

Table S5. Glycan composition of Env and antibody constructs, related to Figures 1, 2 and Figure S3.

Glycoprotein	Abundance of $Man_{5-9}GlcNAc_2$ (labelled $M_{5-9}N_2$) (%)				
	M_5N_2	M_6N_2	M_7N_2	M_8N_2	M_9N_2
BG505 gp140	15.3	4.1	7.2	31.7	41.7
JR-FL gp140	24.6	5.8	10.3	25.6	33.7
X1193.c1 gp140	30.4	6.2	8.4	26.8	28.3
PGT122 Fab	>99	-	-	-	-
VRC01 scFv	>99	-	-	-	-

Table S6. Average number of glycan atoms overlapping with the antibodies computed over the trajectory of the three 500 ns simulations, related to Figure 6.

MD Man$_5$-GlcNAc$_2$

Glycan	N88	N133	N137	N156	N160	N187C	N187F	N197	N234	N262	N276	N295	N301	N332	N339	N355	N363	N386	N392	N398	N406	N411	N448	N462	N611	N618	N625	N637
PG9	0	0	3	61	41	13	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGT122	0	0.2	50	0.7	0	0	0	0	0	0	0	0.1	0.7	39	0	0	0	0	0.5	0	0	1	0	0	0	0	0	0
PGT135	0	8.8	13	0	0	0	0	0	0	0	0	0	0	25	13	0	2.7	21	23	0.3	2.8	33	0	0	0	0	0	0
b12	0	25	8	1.1	0.2	63	7.8	77	0	27	0	1.8	51	4.4	0	0	14	9	0	0	0	1.5	0.2	1.8	0	0	0	0
CH103	0	17	1	0.4	0	58	0.9	76	0	0.1	5	0	16	0	0	0	41	5	3	0.2	0	0	0	9	0	0	0	0
HJ16	0	0.6	0	0	0	3.4	0	4.1	0	0	14	0	0	0	0.2	0	27	6.3	8.2	1.7	0	0	0	16	0	0	0	0
VRC01	0	0	0	0	0	4.4	0	19	0.7	0.6	56	0	1.2	0	0	0	4.4	0	0	0.2	0	0	0	32	0	0	0	0.6
VRC13	0	37	3.1	0.4	0	58	1.5	73	0	1.8	2.3	0.1	24	0	0	0	61	26	0.1	0	0	0	0	4.9	0	0	0	0
CD4	0	0	0	0	0	3.9	0	16	0	0	1.6	0	0.5	0	0	0	2.2	0	0	0	0	0	0	6.9	0	0	0	0
PGT151	0	0	0	0	0	0	0	0	0	0.6	0	1.7	0	0	0	0	0	0	0	0	0	0	23	0	3.4	0	0	2.5
8ANC195	0	0	0	0	0	0	0	0	54	0	46	0	0	0	0	1.1	0	0	0	0	0	0	0	27	20	16	1.2	58
35O22	24	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0.6	0	0	0	0	0	0	0	0.2	0	38	74	1.3

MD Man$_7$-GlcNAc$_2$

Glycan	N88	N133	N137	N156	N160	N187C	N187F	N197	N234	N262	N276	N295	N301	N332	N339	N355	N363	N386	N392	N398	N406	N411	N448	N462	N611	N618	N625	N637
PG9	0	0.2	7	76	57	37	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGT122	0	0.3	37	1.3	0	0	0.6	0	0	0	0	7.6	0	48	0	0	0	0	0.5	0	0	3.2	0	0	0	0	0	0
PGT135	0	22	7.1	0	0	0	1.9	0	0	0	0	0.1	0	51	25	0	4.6	18	39	0	4.5	15	0	0	0	0	0	0
b12	0	12	32	12	0	74	7.3	99	0	44	0	40	84	4	0	0	18	13	0.2	0	0	7.1	0.9	0	0	0	0	0
CH103	0	6.2	11	6.7	0	52	4.2	93	0	1.5	0.8	0	44	0	0	0	55	14	3	0.3	0	0	0	1	0	0	0	0
HJ16	0	0	0	0	0	0	0.5	12	0	0	6.3	0	0.7	0	0.2	0	45	11	8.4	3.9	0	0	0	2.7	0	0	0	0
VRC01	0	0	0	0	0	0.3	0	13	1.7	1.7	54	0	17	0	0	0	8.6	0.5	0.2	2	0	0	0	9.1	0	0	0	1.4
VRC13	0	17	18	4	0	38	7.2	104	0	6.8	0.2	11	67	0	0	0	51	43	0.7	0	0	0.3	0	0	0	0	0	0
CD4	0	0	1	0	0	0.1	0	13	0	0	0.2	0	13	0	0	0	3.5	0.4	0	0	0	0	0	0	0	0	0	0
PGT151	0	0	0	0	0	0	0	0	0	2.7	0.3	1.5	0	0	0	0	0	0	0	0	0	0.1	30	0	8.8	0	0	8.2
8ANC195	0	0	0	0	0	0	0	0	57	0	75	0	0	0	0	0.6	0	0	0	0.2	0	0	0	30	31	28		69
35O22	21	0	0	0	0	0	0	0	18	0	0.1	0	0	0	0	0.6	0	0	0	0	0	0	0	0	0	44	80	1.2

MD Man$_9$-GlcNAc$_2$

Glycan	N88	N133	N137	N156	N160	N187C	N187F	N197	N234	N262	N276	N295	N301	N332	N339	N355	N363	N386	N392	N398	N406	N411	N448	N462	N611	N618	N625	N637
PG9	0	0.5	16	92	63	52	66	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGT122	0	0	32	1.5	0	0.3	0	0	0	0	0	1	0.1	55	0	0	0	0.4	6	0	0	0.4	0	0	0	0	0	0
PGT135	0	4.3	37	0	0	0	0	0	0	0	0	0.2	0	72	17	0	4.9	37	74	0.1	4.3	8.5	0	0	0	0	0	0
b12	0	64	7.1	17	0.7	61	39	119	0	51	0	2.1	105	3.1	0	0	29	14	0	0	0	1	0.2	0	0	0	0	0
CH103	0	50	2.3	9.8	0	54	33	121	0	3.2	2	0	65	0	0.3	0	80	17	0.8	0.2	0	0	0	1.9	0	0	0	0
HJ16	0	3.1	0	0	0	12	1.3	20	0	0	11	0	0.7	0	2	0	69	17	0.8	1.9	0	0	0	8.8	0	0	0	1.2
VRC01	0	0.1	0	0	0	11	0.7	31	4.1	2.5	83	0	29	0	0	0	22	0.9	0	0.4	0	0	0.1	20	0	0	0	1.2
VRC13	0	75	2.5	5.7	0	54	33	126	0	4.1	0.7	0.4	72	1.3	0	0	79	46	0.7	0	0	0	0	0	0	0	0	0
CD4	0	0.2	0	0	0	10	0.6	29	0	0	0.7	0	17	0	0	0	11	0.1	0	0	0	0	0	0.2	0	0	0	0.1
PGT151	0	0	0	0	0	0	0	0	0.1	0.8	1.3	12	0	0	0	0	0	0	0	0.2	1	56	0	11	0.4	0	0	11
8ANC195	0	0	0	0	0	0	0	0	89	0	78	0	0	0	0	3.2	0	0	0	0.5	0	0	0	60	7.8	13	29	85
35O22	40	0	0	0	0	0	0	0	9	0	0	0	0	0	0	12	0	0	0	0	0	0	0	4.4	0	42	100	2.9

SUPPLEMENTAL REFERENCES

Adams, P.D., Gopal, K., Grosse-Kunstleve, R.W., Hung, L.W., Ioerger, T.R., McCoy, A.J., Moriarty, N.W., Pai, R.K., Read, R.J., Romo, T.D.*, et al.* (2004). Recent developments in the PHENIX software for automated crystallographic structure determination. J Synchrotron Radiat *11*, 53-55.

Afonine, P.V., Moriarty, N.W., Mustyakimov, M., Sobolev, O.V., Terwilliger, T.C., Turk, D., Urzhumtsev, A., and Adams, P.D. (2015). FEM: feature-enhanced map. Acta Crystallogr D Biol Crystallogr *71*, 646-666.

Agirre, J., Davies, G., Wilson, K., and Cowtan, K. (2015). Carbohydrate anomalies in the PDB. Nature chemical biology *11*, 303.

Behrens, A.J., Vasiljevic, S., Pritchard, L.K., Harvey, D.J., Andev, R.S., Krumm, S.A., Struwe, W.B., Cupo, A., Kumar, A., Zitzmann, N., *et al.* (2016). Composition and antigenic effects of individual glycan sites of a trimeric HIV-1 envelope glycoprotein. Cell Rep. *S2211-1247*, 30179-30176.

Best, R.B., Zhu, X., Shim, J., Lopes, P.E., Mittal, J., Feig, M., and Mackerell, A.D., Jr. (2012). Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone phi, psi and side-chain chi(1) and chi(2) dihedral angles. Journal of chemical theory and computation *8*, 3257-3273.

Binley, J.M., Sanders, R.W., Clas, B., Schuelke, N., Master, A., Guo, Y., Kajumo, F., Anselma, D.J., Maddon, P.J., Olson, W.C.*, et al.* (2000). A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. J Virol *74*, 627-643.

Blixt, O., Head, S., Mondala, T., Scanlan, C., Huflejt, M.E., Alvarez, R., Bryan, M.C., Fazio, F., Calarese, D., Stevens, J.*, et al.* (2004). Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci U S A *101*, 17033-17038.

Bricogne, G.B., E.; Brandl, M.; Flensburg, C.; Keller, P.; Paciorek, W.; Roversi, P.; Sharff, A.; Smart, OS.; Vonrhein, C.; Womack, T. O. (2011). BUSTER version 2.10.0. . Cambridge, UK: Global Phasing Ltd.

Chen, B., Vogan, E.M., Gong, H., Skehel, J.J., Wiley, D.C., and Harrison, S.C. (2005). Structure of an unliganded simian immunodeficiency virus gp120 core. Nature *433*, 834-841.

Core, T.R. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Craig, D.B., and Dombkowski, A.A. (2013). Disulfide by Design 2.0: a web-based tool for disulfide engineering in proteins. BMC Bioinformatics *14*, 346.

Davis, I.W., Murray, L.W., Richardson, J.S., and Richardson, D.C. (2004). MOLPROBITY: structure validation and all-atom contact analysis for nucleic acids and their complexes. Nucleic Acids Res *32*, W615-619.

Eisenhaber, F.A., P.; (1993). Improved strategy in analytical surface calculation for molecular system- handling of singularities and computational efficiency. J Comp Chem *14*, 1272-1280.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr *60*, 2126-2132.

Garces, F., Lee, J.H., de Val, N., Torrents de la Pena, A., Kong, L., Puchades, C., Hua, Y., Stanfield, R.L., Burton, D.R., Moore, J.P*., et al.* (2015). Affinity Maturation of a Potent Family of HIV Antibodies Is Primarily Focused on Accommodating or Avoiding Glycans. Immunity *43*, 1053-1063.

Garces, F., Sok, D., Kong, L., McBride, R., Kim, H.J., Saye-Francisco, K.F., Julien, J.P., Hua, Y., Cupo, A., Moore, J.P*., et al.* (2014). Structural evolution of glycan recognition by a family of potent HIV antibodies. Cell *159*, 69-79.

Georgiev, I.S., Doria-Rose, N.A., Zhou, T., Kwon, Y.D., Staupe, R.P., Moquin, S., Chuang, G.Y., Louder, M.K., Schmidt, S.D., Altae-Tran, H.R*., et al.* (2013). Delineating antibody recognition in polyclonal sera from patterns of HIV-1 isolate neutralization. Science *340*, 751-756.

Guvench, O., Mallajosyula, S.S., Raman, E.P., Hatcher, E., Vanommeslaeghe, K., Foster, T.J., Jamison, F.W., 2nd, and Mackerell, A.D., Jr. (2011). CHARMM additive all-atom force field for carbohydrate derivatives and its utility in polysaccharide and carbohydrate-protein modeling. Journal of chemical theory and computation *7*, 3162-3180.

Harvey, M.J., Giupponi, G., and Fabritiis, G.D. (2009). ACEMD: Accelerating Biomolecular Dynamics in the Microsecond Time Scale. Journal of chemical theory and computation *5*, 1632-1639.

Jorgensen, W.C., J.; Madura, J.; Impey, R.; Klein, M. (1983). Comparison of simple potential functions for simulating liquid water. J Chem Phys *79*, 926-935.

Julien, J.P., Lee, J.H., Cupo, A., Murin, C.D., Derking, R., Hoffenberg, S., Caulfield, M.J., King, C.R., Marozsan, A.J., Klasse, P.J*., et al.* (2013). Asymmetric recognition of the HIV-1 trimer by broadly neutralizing antibody PG9. Proc Natl Acad Sci U S A *110*, 4351-4356.

Kong, L., Kadam, R.U., Giang, E., Ruwona, T.B., Nieusma, T., Culhane, J.C., Stanfield, R.L., Dawson, P.E., Wilson, I.A., and Law, M. (2015). Structure of Hepatitis C Virus Envelope Glycoprotein E1 Antigenic Site 314-324 in Complex with Antibody IGH526. J Mol Biol *427*, 2617-2628.

Lee, J.E., Fusco, M.L., Hessell, A.J., Oswald, W.B., Burton, D.R., and Saphire, E.O. (2008). Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature *454*, 177-182.

Majeed, S., Ofek, G., Belachew, A., Huang, C.C., Zhou, T., and Kwong, P.D. (2003). Enhancing protein crystallization through precipitant synergy. Structure *11*, 1061-1070.

McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., and Read, R.J. (2007). Phaser crystallographic software. Journal of applied crystallography *40*, 658-674.

McLellan, J.S., Chen, M., Joyce, M.G., Sastry, M., Stewart-Jones, G.B., Yang, Y., Zhang, B., Chen, L., Srivatsan, S., Zheng, A.*, et al.* (2013a). Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science *342*, 592-598.

McLellan, J.S., Chen, M., Leung, S., Graepel, K.W., Du, X., Yang, Y., Zhou, T., Baxa, U., Yasuda, E., Beaumont, T.*, et al.* (2013b). Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. Science *340*, 1113-1117.

McLellan, J.S., Pancera, M., Carrico, C., Gorman, J., Julien, J.P., Khayat, R., Louder, R., Pejchal, R., Sastry, M., Dai, K.*, et al.* (2011). Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature *480*, 336-343.

Meyer, B., and Peters, T. (2003). NMR spectroscopy techniques for screening and identifying ligand binding to protein receptors. Angew Chem Int Ed Engl *42*, 864-890.

Montefiori, D.C. (2009). Measuring HIV neutralization in a luciferase reporter gene assay. Methods Mol Biol *485*, 395-405.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol *276*, 307-326.

Pancera, M., Zhou, T., Druz, A., Georgiev, I.S., Soto, C., Gorman, J., Huang, J., Acharya, P., Chuang, G.Y., Ofek, G.*, et al.* (2014). Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature *514*, 455-461.

Petrey, D., Xiang, Z., Tang, C.L., Xie, L., Gimpelev, M., Mitros, T., Soto, C.S., Goldsmith-Fischman, S., Kernytsky, A., Schlessinger, A.*, et al.* (2003). Using multiple structure alignments, fast model building, and energetic analysis in fold recognition and homology modeling. Proteins *53 Suppl 6*, 430-435.

Pritchard, L.K., Vasiljevic, S., Ozorowski, G., Seabright, G.E., Cupo, A., Ringe, R., Kim, H.J., Sanders, R.W., Doores, K.J., Burton, D.R.*, et al.* (2015). Structural Constraints Determine the Glycosylation of HIV-1 Envelope Trimers. Cell reports *11*, 1604-1613.

Sanders, R.W., Derking, R., Cupo, A., Julien, J.P., Yasmeen, A., de Val, N., Kim, H.J., Blattner, C., de la Pena, A.T., Korzun, J.*, et al.* (2013). A next-generation cleaved, soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog *9*, e1003618.

Sanders, R.W., Vesanen, M., Schuelke, N., Master, A., Schiffner, L., Kalyanaraman, R., Paluch, M., Berkhout, B., Maddon, P.J., Olson, W.C.*, et al.* (2002). Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. J Virol *76*, 8875-8889.

Shivatare, S.S., Chang, S.-H., Tsai, T.-I., Tseng, S.Y., Shivatare, V.S., Lin, Y.-S., Cheng, Y.-Y., Ren, C.-T., Lee, C.-C.D., Pawar, S.*, et al.* (2016). Modular synthesis of N-glycans and arrays for the hetero-ligand binding analysis of HIV antibodies. Nat Chem *Published online March 07, 2016*.

Shivatare, S.S., Chang, S.H., Tsai, T.I., Ren, C.T., Chuang, H.Y., Hsu, L., Lin, C.W., Li, S.T., Wu, C.Y., and Wong, C.H. (2013). Efficient convergent synthesis of bi-, tri-, and tetra-antennary complex type N-glycans and their HIV-1 antigenicity. J Am Chem Soc *135*, 15382-15391.

Soto, C.S., Fasnacht, M., Zhu, J., Forrest, L., and Honig, B. (2008). Loop modeling: Sampling, filtering, and scoring. Proteins *70*, 834-843.

Stevens, J., Blixt, O., Tumpey, T.M., Taubenberger, J.K., Paulson, J.C., and Wilson, I.A. (2006). Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. Science *312*, 404-410.

Szakonyi, G., Klein, M.G., Hannan, J.P., Young, K.A., Ma, R.Z., Asokan, R., Holers, V.M., and Chen, X.S. (2006). Structure of the Epstein-Barr virus major envelope glycoprotein. Nat Struct Mol Biol *13*, 996-1001.

Terwilliger, T.C., Grosse-Kunstleve, R.W., Afonine, P.V., Moriarty, N.W., Zwart, P.H., Hung, L.W., Read, R.J., and Adams, P.D. (2008). Iterative model building, structure refinement and density modification with the PHENIX AutoBuild wizard. Acta Crystallogr D Biol Crystallogr *64*, 61-69.

Wu, X., Parast, A.B., Richardson, B.A., Nduati, R., John-Stewart, G., Mbori-Ngacha, D., Rainwater, S.M., and Overbaugh, J. (2006). Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. J Virol *80*, 835-844.

Wu, X., Yang, Z.Y., Li, Y., Hogerkorp, C.M., Schief, W.R., Seaman, M.S., Zhou, T., Schmidt, S.D., Wu, L., Xu, L.*, et al.* (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science *329*, 856-861.

Xiang, Z., and Honig, B. (2001). Extending the accuracy limits of prediction for side-chain conformations. J Mol Biol *311*, 421-430.

Zhang, Y., and Skolnick, J. (2005). TM-align: a protein structure alignment algorithm based on the TM-score. Nucleic Acids Res *33*, 2302-2309.